Salivary Gland Hypertrophy Viruses: A Novel Group of Insect Pathogenic Viruses

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Key Words

insect DNA virus, *Hytrosaviridae*, Diptera, pathology, sterilizing effect, sterile insect technique

Abstract

Salivary gland hypertrophy viruses (SGHVs) are a unique, unclassified group of entomopathogenic, double-stranded DNA viruses that have been reported from three genera of Diptera. These viruses replicate in nuclei of salivary gland cells in adult flies, inducing gland enlargement with little obvious external disease symptoms. Viral infection inhibits reproduction by suppressing vitellogenesis, causing testicular aberrations, and/or disrupting mating behavior. Historical and present research findings support a recent proposal of a new virus family, the *Hytrosaviridae*. This review describes the discovery and prevalence of different SGHVs, summarizes their biochemical characterization and taxonomy, compares morphological and histopathological properties, and details transmission routes and the influence of infection on host biology and reproduction. In addition, the potential use of SGHVs as sterilizing agents for house fly control and the deleterious impact of SGHVs on colonized tsetse flies reared for sterile insect technique are discussed.

SGH: salivary gland hypertrophy

SGHV: salivary gland hypertrophy virus

DISCOVERYS AND SIGNIFICANCE OF SGHV

The few described viruses associated with symptoms of salivary gland hypertrophy (SGH) in adult dipteran insects were discovered in the early 1970s. The need to dissect insects to detect hypertrophied glands may explain, in part, the limited number of known insect species harboring the salivary gland hypertrophy viruses (SGHVs). To date, there is only one report of SGHV infection in populations of the adult narcissus bulb fly, *Merodon equestris* (Diptera: Syrphidae) (8, 56). A survey conducted in southern France in the early 1970s revealed high incidences of SGH in flies from two varieties of this insect species. SGH was recorded in 31% and 54% of adult *M. equestris* var. *nobilis* and *M. equestris* var. *transversalis*, respectively; this symptom was accompanied by atrophied gonads in both genders (56). Long, rod-shaped virus particles isolated from the hypertrophied salivary glands showed ultrastructural similarities to certain baculoviruses and were speculated to cause SGH (8).

The first description of SGHV in tsetse flies (Diptera: Glossinidae) also dates to the 1970s, when Jenni & Steiger (37) published results from their ultrastructural examination of trypanosome development in the salivary glands of adult *Glossina morsitans centralis* collected from Singida, United Republic of Tanzania. On the basis of morphological characteristics of the detected virus particles, the authors suggested a resemblance to arboviruses (37), an incorrect, misleading association. Several years later, virus particles were discovered in nuclei, cytoplasm, intercellular spaces, and lumina of enlarged salivary glands of adult *G. pallidipes* collected from Kibwezi Forest, Kenya (34). In these collections, the percentage of flies with SGH symptoms was low and varied between 1% and 2%. A significant proportion of the symptomatic *G. pallidipes* displayed atrophied gonads in both genders, indicating a sterilizing effect of SGHV infection on the tsetse host (34). SGHV infections recently have been linked to the collapse of valuable colonies of *G. pallidipes* at the Insect Pest Control Laboratory (former Entomology Unit) of the FAO/IAEA Joint Program in Seibersdorf, Austria (1). Following these discoveries, SGHVs from eight *Glossina* species collected from seven different African countries have been described (**Table 1** and references therein).

In the 1990s, Coler et al. (15) discovered a third dipteran genus harboring SGHV. In dissections of adult house flies, *Musca domestica* (Diptera: Muscidae), sampled from populations in Florida for a survey of parasitic nematodes, several flies contained grossly enlarged, discolored, whitish-blue salivary glands. Histological and biochemical examination revealed that a rod-shaped, double-stranded DNA (dsDNA) virus was associated with these symptomatic glands, and the authors pointed out the striking similarity between this virus and the above described SGHVs. Most females (95%) displaying SGH had undeveloped ovaries, again demonstrating a sterilizing effect of viral infection (15).

Over the past four decades, several research groups in Europe, Africa, and the United States have investigated the SGHVs infecting tsetse flies and house flies to identify ultrastructural, biochemical, and molecular characteristics, pathology, transmission, field incidence, and geographical distribution of these viruses. The results are summarized and discussed in this review.

CHARACTERIZATION AND TAXONOMY OF SGHV

Structure and Composition

Transmission electron microscopy (TEM) examination of ultrathin sections of hypertrophied salivary glands from different host flies revealed the presence of numerous rod-shaped virus particles in the nucleus, cytoplasm, intercellular spaces, and gland lumen (**Figure 1*a***) (34). Nucleocapsids assemble in the nuclei and aggregate in spaces adjacent to the virogenic stroma, the presumed site of DNA replication. The size of the nucleocapsids varies among the different SGHVs; the *Musca domestica* SGHV

Table 1 Reported host species of SGHVs and prevalence of SGH symptoms in host populations

Species	Location	Prevalence (%)[a]	Reference
Glossina austeni Newstead	Tanzania (Amani)	1.6 (432)	13[b,c]
G. morsitans Westwood	Tanzania (Singida, Kondoa)	0.1 (8,916)	13[b,d]
G. m. centralis Machado	Tanzania (Singida)	No data	36,[e] 37
G. m. morsitans Westwood	Zimbabwe (Zambezi valley)	0.5 (1,162)	18
G. m. morsitans Westwood	Lab colony, Kenya (Nairobi)	No data	50
G. nigrofusca nigrofusca Newstead	Ivory Coast (Vavoua)	0.7 (143)	28
G. pallicera pallicera Bigot	Ivory Coast (Vavoua)	2.1 (287)	28
G. pallidipes Austen	Kenya (East coast, various sites)	2.7 (17,180)	62
G. pallidipes Austen	Kenya (Kiboko)	0.4 (23,960)	59
G. pallidipes Austen	Kenya (Kibwezi forest)	1.0 (5,361) in 1975/1976; 1.2 (491) in 1980	34, 67
G. pallidipes Austen	Kenya (Lambwe Valley Game Reserve)	1.6 (929)	40, 41, 51, 67
G. pallidipes Austen	Kenya (Meru National Park)	0.9 (439)	67
G. pallidipes Austen	Kenya (Mombasa)	1.8 (18,410)	64
G. pallidipes Austen	Kenya (Shimba Hills Game Reserve)	5.4 (204)	67
G. pallidipes Austen	Kenya (Sindo)	1.1 (8,403)	59
G. pallidipes Austen	Kenya (South coast, primary forest)	7.0 (1,213)	63
G. pallidipes Austen	Kenya (South coast, secondary forest)	3.8 (662)	63
G. pallidipes Austen	Kenya (South coast, shrub area)	3.2 (464)	63
G. pallidipes Austen	Kenya (South coast, fallow land)	1.8 (329)	63
G. pallidipes Austen	South Africa (Zululand)	2.9 (1,129)	90, 91[b]
G. pallidipes Austen	Tanzania (Amani)	1.7 (376)	13[b]
G. pallidipes Austen	Zimbabwe (Zambezi valley)	2.0 (886)	18
G. pallidipes Austen	Laboratory colony, Austria (Seibersdorf); origin: Ethiopia (Southern Rift Valley)	>85.0 (unknown)	1
G. pallidipes Austen	Laboratory colony, Austria (Seibersdorf); origin: Uganda (Tororo)	3.8 (2,011)	1, 4
G. palpalis palpalis Rob. Desv.	Ivory Coast (Vavoua)	0.3 (1,351)	28
Musca domestica L.	California	2.0 (100)	71
Musca domestica L.	Denmark (Havbro)	1.0 (100)	71
Musca domestica L.	Denmark (Morum)	1.0 (200)	71
Musca domestica L.	Denmark (Slangerup)	1.0 (200)	71
Musca domestica L.	Denmark (Tønder)	4.7 (169)	71
Musca domestica L.	Florida (various sites)	6.3 (11,110) in 1991 0.5–10.0 (28,800) in 2005/2006	15, 24, 71
Musca domestica L.	Kansas	0.7 (155)	71
Musca domestica L.	New Zealand	No data	71
Musca domestica L.	Thailand	11.1 (45) in 2008; 1.8 (110) in 2009	71
Musca domestica L.	Virgin Islands	1.2 (276)	71
Merodon equestris F.	France	30.8 (39) and 54.1 (37)	8, 56

[a]Numbers in parentheses indicate total numbers of flies dissected.
[b]Correlation with virus infection not established in these articles.
[c]Statements in the article are contradictory: Text states no SGH in this species, whereas table indicates 7 of 432 flies with SGH.
[d]Tables in the article are contradictory: One table indicates no SGH in this species, another table and the text state 7 of 8,916 flies with SGH.
[e]No salivary gland enlargement described in this study.

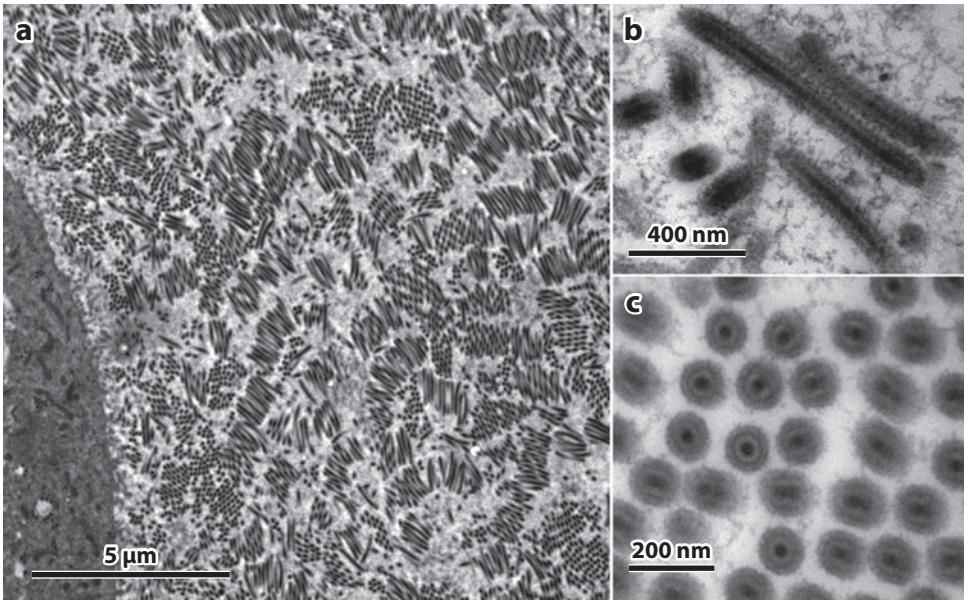

Figure 1

Transmission electron micrographs of *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV). (*a*) Luminal region of tsetse fly salivary gland displaying the numerous enveloped GpSGHV virions. High magnification images of the enveloped GpSGHV in (*b*) longitudinal section and (*c*) cross section demonstrate the complex structure of the particles.

(MdSGHV) and the *Merodon equestris* SGHV (MeSGHV) measure ~500–600 nm in length by 50–60 nm in diameter, whereas the nucleocapsids associated with tsetse fly SGHVs are significantly longer and measure 800–1200 nm in length by 50–60 nm in diameter (**Figure 1*b***) (8, 24, 34). Negative staining revealed that the nucleocapsids comprise structural units arranged as a series of stacked rings 7 to 10 nm wide (18, 64). The nucleocapsids exit the nuclei via nuclear pores, associate with the Golgi apparatus, and acquire their envelope in situ in the cytoplasm. Enveloped virus particles, measuring 70–80 nm in diameter, consist of an inner membrane that encloses the nucleocapsid and an outer membrane separated from the inner membrane by a narrow space (**Figure 1*c***) (34). Both the *Glossina pallidipes* SGHV (GpSGHV) and the MdSGHV band at a density of 1.153 g cm^{-3} when subjected to 10–60% Nycodenz® gradient centrifugation (22).

Biochemical analysis demonstrated that the SGHVs contain a complex array of major and minor structural proteins. SDS-PAGE analysis of the Ugandan GpSGHV isolate revealed more than 35 protein bands ranging in size from 10 to 220 kDa (3). At least six bands are larger than 100 kDa, with the major bands having a molecular mass of 39 and 40 kDa. A similar analysis conducted on Nycodenz-purified MdSGHV revealed a complex of major and minor bands that range from 10 to 200 kDa (15, 22). SDS-PAGE and nanocapillary liquid chromatography tandem mass spectrometry (GeLC-MS/MS) analysis of MdSGHV peptides separated on SDS gels identified unique peptide fragments that were encoded on 29 open reading frames (ORFs) (22). Sixteen of the MdSGHV structural ORFs have homologs detected in the GpSGHV genome, whereas only four ORFs are homologous to non-SGHV genes (21, 22). No protein inclusions or viral

occlusions have been detected in SGHV-infected cells.

Genome Organization

Early work demonstrated that the MdSGHV contained a relatively large (>100 kbp) single dsDNA molecule (15). The detection of two bands (supercoiled and relaxed forms) after agarose electrophoresis of purified DNA and the lack of end-labeling of undigested DNA indicated SGHVs possessed circular genomes. The genomes of both the GpSGHV (NC_010356.1) and the MdSGHV (NC_010671) have been fully sequenced by a combination of conventional sequencing of viral clones and pyrosequencing (3, 22). The longer GpSGHV encapsidates a 190,032-bp genome (28% G + C), whereas the smaller MdSGHV virion encapsidates a 124,279-bp genome (44% G + C). Both genomes were derived from wild-type virus isolates, and attempts to replicate SGHVs in insect cell cultures have failed, precluding access to clonal preparations. Analysis of sequence data demonstrated the presence of polymorphic sites involving single-base substitutions located randomly throughout the genome (22).

In silico analysis demonstrated that the GpSGHV genome encodes for 322 potential ORFs for proteins composed of at least 50 amino acids with a methionine start codon (3). Of these, only 160 ORFs possess either no or minimal overlap with adjacent ORFs (3). The predicted 160 ORFs, representing 86% of the genome, are distributed evenly on both strands (51% forward and 49% reverse) with many arranged in unidirectional gene clusters. A total of 108 putative MdSGHV ORFs were identified in silico (22). The transcriptional orientation of the predicted ORFs was slightly different, with 53 ORFs (49%) in the clockwise direction and 55 (51%) in the opposite direction. Similar to the GpSGHV, several clusters of MdSGHV ORFs were transcribed in one direction. The majority of ORFs identified on the two SGHV genomes have no detectable homologs when subjected to BLAST analysis (3, 21, 22). For example, only 30 and 47 of the 108 and 160 putative ORFs detected in the MdSGHV and GpSGHV, respectively, could be assigned to any homolog. The identified homologs encode for structural proteins, proteins involved in DNA replication (e.g., DNA polymerase, helicase), and protein-modifying enzymes (e.g., protein kinase). A total of 101 MdSGHV ORFs have been validated using rapid amplification of cDNA 3′ ends and reverse transcriptase PCR (73). Most are transcribed as individual transcripts, whereas 34 ORFs are transcribed in tandem with adjacent ORFs (73); similar events have occurred in other insect dsDNA viruses (20, 30, 69). Analysis of the MdSGHV 3′-untranslated regions (3′-UTRs) revealed extensive heterogeneity in both the polyadenylation signals and cleavage sites present on the MdSGHV ORFs (73). Convergent, unidirectional, and divergent overlap found in the 3′-UTRs of 34 transcript pairs suggests *cis*-encoded natural antisense viral transcription (82). Promoter analysis revealed that the 5′ upstream regions in both the MdSGHV and the GpSGHV are highly enriched with a TAAG motif, which is identical to the canonical baculovirus late transcription initiation sequence (3, 22). In addition to the ORFs and their associated UTRs, a series of direct repeats (drs) is distributed throughout the GpSGHV (14 drs) and MdSGHV (18 drs) genomes.

Classification of *Hytrosaviridae*

When discovered, the SGHVs were tentatively associated with arboviruses and the nonoccluded baculoviruses (formerly subgroup C baculoviruses, presently nudiviruses) (34, 37). At that time, available morphological (enveloped, rod-shaped), molecular (large circular dsDNA genome), and biological (oral infectivity, nuclear replication) information justified placement of the SGHVs with the nudiviruses. However, as sequence information became available (2, 24), the relationships between the SGHVs and other dsDNA insect viruses

Hypertrophy: enlargement or overgrowth of an organ or part of the body due to the increased size of the constituent cells

became less defined. Gene parity plot analyses demonstrated colinear regions between the MdSGHV and the GpSGHV but failed to display any linear correspondence between the SGHVs and the sequenced nudiviruses GbNV and HzNV-1 (21). Furthermore, syntenic map analysis displayed comparable colinearity between the MdSGHV and GpSGHV genomes, which was not found when either genome was compared to GbNV or HzNV-1. Finally, phylogenetic analysis of selected genes (e.g., DNA polymerase, per os infectivity factors) failed to show any association with homologs from other dsDNA insect viruses (21). These genetic differences, in combination with the unique (patho)biological properties displayed by SGHVs, justify the proposal of a new virus family named *Hytrosaviridae* (2). The SGHVs infect and replicate in adult flies and cause distinct SGH symptoms that result in insect sterility. Although the GpSGHV and MdSGHV share general relatedness in some characters, they possess many unique properties suggesting separate genera, the *Glossinavirus* and *Muscavirus*, respectively.

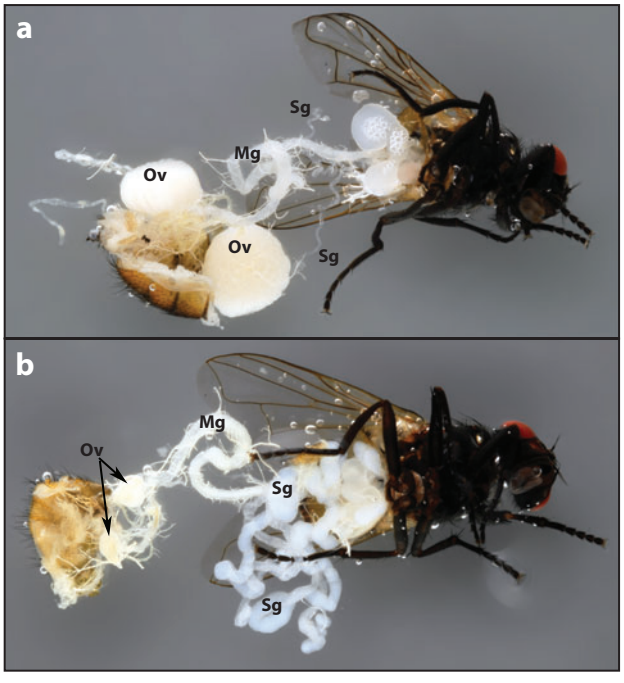

Figure 2

Musca domestica females with (*a*) healthy and (*b*) hypertrophied salivary glands showing the lack of ovarian development in the SGHV-infected fly (*b*). Abbreviations: Mg, midgut; Ov, ovary; Sg, salivary gland.

PATHOLOGICAL EFFECTS OF SGHV

External Morphology of the Infected Host

Although SGHV infection enlarges salivary glands, which eventually fill most of the abdominal cavity, flies with SGH cannot be distinguished easily from normal flies by external visual examination (62). However, in teneral tsetse flies, the enlarged, bluish-white salivary glands may appear as a pale outline through the abdominal integument and form irregular ridges on the soft cuticle. In addition, a bloated abdomen in *G. pallidipes* males that contains traces of blood two days after feeding suggests the presence of SGH (4). In older flies, pigmentation, thickening of the cuticle, or enlargement of the abdomen in gravid females precludes detection of SGH.

Impact of SGHV Infection on Salivary Glands

Morphological characteristics of SGH were described first by Whitnall in 1932: Enlarged salivary glands of *G. pallidipes* were swollen to almost four times their normal thickness (90, 91). During dissections of the various hosts, hypertrophied glands are distinguishable from normal, transparent salivary glands due to their large size and chalky-white or bluish appearance (**Figure 2**) (1, 8, 15, 28, 54). Typically, the paired salivary glands are equally affected, and hypertrophy is uniform over the entire length of the distal part (15, 62). Whereas the diameter of the distal part of a normal salivary gland is about 100 μm with almost no variation throughout the life of the tsetse fly, the diameter of hypertrophied salivary glands increases to up to 1.1 mm and has been used to categorize glands with SGH symptoms into nine size classes (62). In the smallest

size category, i.e., at an early stage of disease, discoloration without enlargement is observed because epithelial cells expand toward the gland lumina, resulting in a constricted lumen. In hypertrophied glands of category 9, the entire abdominal hemocoel is filled with grossly enlarged and highly coiled salivary glands. Late-stage hypertrophied glands are about twice as long as normal salivary glands (62).

Ultrastructural studies have shown that in tsetse flies and in the narcissus bulb fly, gland enlargement is caused by cellular proliferation of the glandular epithelial cells and hypertrophy of their nuclei and cytoplasm, resulting in an abnormal multilayered epithelium and a reduced gland lumen (8, 34, 50, 67). In contrast, SGH in house flies appears to be caused by nuclear and cellular hypertrophy without any increase of cell numbers. In all known SGHV hosts, diseased salivary glands develop heavily vacuolated cytoplasm, cell membranes separate from adjacent epithelial cells, and numerous rod-shaped virus particles are observed in the nuclei, cytoplasm, intercellular spaces, and gland lumina (8, 24, 34, 67). The presence of both nucleocapsids and enveloped virions in the cytoplasm of hypertrophied salivary gland cells indicates that the SGHVs assemble their envelope in the cytoplasm (18, 24).

It is not known whether the enzyme composition and/or functions of hypertrophied salivary glands are negatively affected by the disease. The observed histopathological aberrations suggest that feeding and digestion may be impaired in diseased flies, and several studies have examined the impact of SGHV infection on fitness and on survival rates (see below). The long survival time of infected flies indicates that salivary gland function is maintained.

Tissue Tropism and Impact of the Virus on Other Tissues of Infected Flies

The primary tissue infected with SGHV is the salivary gland, but the virus is also present in other tissues of infected flies as demonstrated by TEM (40, 41, 49, 55, 76), diagnostic PCR (1, 4, 54), and infection bioassays (54, 55). In TEM studies, virus particles have been observed in the crop and midgut lumen (55, 77), in intercellular spaces of muscle tissue (55), in nuclei and cytoplasm of milk gland cells (76), in germ cell nuclei and nurse cell and oocyte cytoplasm of ovarioles (40, 41), and in the lumen and epithelial cells of male accessory reproductive glands (ARGs) (49). Detection of both virogenic stroma and associated nucleocapsids within nuclei is proof of viral replication in ovarian germ cells, follicles, and milk gland cells (40, 76). In several reports, virions were not detected in testes, male ARGs, flight muscle, fat body, and spermathecae of SGH-positive flies (67, 75). Detection of viral DNA by PCR in excised legs indicated the presence of hemolymph-borne virus (1). Bioassays confirmed that virions detected by PCR in crops and ovaries of viremic flies were infectious and induced SGH when delivered orally or through intrahemocoelic injection (54, 55).

The most obvious impact of SGHV infection on nonsalivary gland tissues is the abnormal development of gonads (40, 54, 56). Ovaries of SGHV-infected house flies remain at the previtellogenic stage (**Figure 2**), and the expression of the female-specific hexamerin and yolk protein genes by fat body cells is downregulated (54). Infected tsetse females display irregular ovariolar development (34) or severe necrosis and degeneration of the germaria (40). Examination of testes from viremic tsetse flies indicated a complete arrest of spermatogenesis, with follicles containing highly vacuolated, degenerate spermatogenic cells (40); these males did not produce spermatophores (75). Although virus particles may not be detected in male ARGs, these tissues can be affected by SGHV infection of the insect, showing reduction in size, disintegration of epithelial cell organelles, and detachment of adjacent muscle cells from the basal lamina (75). In some cases, viremic flies show midgut hypertrophy (62) and midgut epithelial cell necrosis, degeneration, and lysis in the anterior, secretory, and posterior parts (77). Presumably, secretory and absorptive functions of the midgut are

impaired, which, in concert with dysfunctional salivary glands, decreases nutrient assimilation and leads to starvation (77). Virus particles also were detected in the milk glands of female tsetse flies (*G. morsitans centralis*) with SGH (76). Viral replication and severe necrotic lesions in the secretory cell layers of these glands suppress milk synthesis, which hinders F_1 larval development and decreases viability (76).

Impact of Viral Infection on Host Fitness and Behavior

Susceptibility/resistance to infection. Typically, injection with viral preparations results in 100% symptomatic infection within several days after injection (42, 54, 71). While tsetse flies may be injected as larvae to produce infected adults (42, 75), only adults have been used for injection experiments with house flies (54, 71). Per os infectivity of the MdSGHV to newly emerged house flies varies between 2% and 83% (24, 55, 71); the origin of viral inoculum (salivary glands, crops, saliva, feces) as well as differences in viral titers between inocula may explain this variation. Significantly, adult house flies develop resistance to oral infection within hours after eclosion; *per os* treatment of 24-h-old and 2-h-old flies with the same MdSGHV preparation yields, on average, sixfold-lower infection rates in the older flies (71). The factors responsible for age-related resistance to MdSGHV infection, although unknown, could be related to the maturation of the peritrophic matrix.

Mortality. Laboratory tsetse and house flies with SGH can survive for at least two weeks, often longer (15, 35, 54, 77). The few studies examining survival rates have reported either cumulative mortality or mean life span. The detection of SGH in field-collected tsetse flies that harbored mature trypanosomes, indicating that these flies were at least three weeks old, suggests that viremic flies survive for several weeks (91). Alternatively, adult field flies may acquire the virus or develop SGH after a period of chronic asymptomatic infection later in their life. Field data have shown that a high proportion of *G. pallidipes* with SGH (63%) are young flies (nulliparous females and teneral males), whereas in the asymptomatic group only 14% are young flies, suggesting either high mortality of infected flies (35) or reduced susceptibility as flies age. Although some data show that infected flies survive as long as healthy flies (15), most reports demonstrate that SGHV infection reduces the life span of the insect. Laboratory healthy and viremic *G. pallidipes* females, for example, have a maximum life span of 112–161 days and 56–58 days, respectively. Similarly, the life span of *G. m. centralis* is significantly reduced in viremic flies (63 and 29 days in heavily infected females and males, respectively) compared with asymptomatic flies (106 and 92 days in females and males, respectively), and the reduction in life span is positively correlated to the severity of infection (77). Cumulative mortality of viremic female *M. domestica* significantly increases to 69% after 16 days compared with 24% mortality of healthy females (54).

Flight and feeding. In the field, tsetse flies with SGH show normal flight behavior, and their blood-filled gut indicates they are capable of securing a blood meal (62). In the laboratory, *G. pallidipes* with SGH feed normally on rabbits (62) or on a membrane-feeding system (5). In contrast, in *G. m. centralis*, SGH impairs the ability to feed: Symptomatic flies probe more often, take more time, and imbibe less blood during feeding than nonsymptomatic flies do (77). These smaller blood meals could be attributed to reduced saliva secretion hampering blood uptake and/or enlarged salivary glands occupying the entire abdominal space and limiting blood ingestion. In addition, *G. m. centralis* with SGH have difficulty digesting blood, as indicated by the presence of blood clots in the crop (77). Blood clotting might be due to insufficient release of saliva in the hypertrophied salivary glands, leading to incomplete blood anticoagulation. The difficulty in feeding on and digesting blood, in combination with the subsequent rupture of the crop and/or midgut, may

explain the high mortality observed in viremic *G. m. centralis*. Multiple attempts to obtain a blood meal from a host by infected tsetse flies may induce host defensive behaviors, leading to interrupted or aborted feeding. House flies with SGH are capable of ingesting and digesting protein-containing food, although proteolytic activity in the midgut is reduced compared with healthy flies (54).

Reproduction. The effect of SGHV infection on the reproductive potential of infected flies appears different within and between various tsetse fly species and house flies. Female *G. pallidipes* with SGH, for example, mate with normal males and produce offspring, whereas the viremic males are mostly unable to inseminate female flies (34). However, significant proportions of both female (45%) and male (71%) *G. pallidipes* with SGH show abnormal gonad development, indicating that SGHV-infection sterilizes both genders (34). In *G. m. morsitans* and *G. m. centralis*, insemination by males with SGH is also impaired (39). Mating behavior of tsetse flies with SGH appears to be normal. Both mating duration and time to reach the jerking phase before separation are similar for healthy and viremic *G. m. morsitans* (39). Although no sperm are transferred to the female spermathecae by male *G. m. morsitans* with SGH, a portion of the females becomes refractory to remating, as is the case when the first mating partner is a normal male (39). Jura & Davies-Cole (39) speculated that males sterilized by SGHV infection retain a competitive mating efficiency and may be useful in a sterile male release program. However, choice assays or field trials have not been conducted to verify this claim. The fecundity of females with SGH is significantly reduced in both tsetse and house flies. Viremic *G. m. centralis* females have longer pregnancy cycles and produce pupae with lower weights than do healthy females (74). In *M. domestica*, SGHV infection completely inhibits vitellogenesis (54). Only females that acquire the virus after completion of the first gonadotropic cycle are able to deposit one fertilized batch of eggs. However, with the onset of SGH symptoms, virgin, egg-containing females become unresponsive to mating attempts and do not copulate (54). Male house flies with SGH are able to copulate and deliver viable sperm, but with progressing infection, their avidity is reduced (54). While sterile insects could be expected to have an extended life span, increased mortality of flies with SGH can be partially explained by impaired digestion.

SGH and Infection with Other Microorganisms

SGH was first observed during prevalence studies of trypanosomes in tsetse flies. Prevalence of SGH positively correlated with that of *Trypanosoma* spp. in *G. pallidipes* but not in *G. morsitans*, *G. p. palpalis*, *G. p. pallicera*, and *G. n. nigrofusa* (13, 28, 67, 91). Jaenson (34) therefore cautioned that the impact of viremia on the vector potential of *G. pallidipes* would need assessment before considering the use of SGHVs as biological control agents against tsetse flies. However, later surveys found no relationship between SGH and trypanosome infection (18, 59). The impact of SGHV infection on trypanosome transmission is unclear (67). Histopathological examination of tsetse salivary glands indicated that SGHV-trypanosome mixed infections caused severe cellular disintegration (cytoplasmic vacuolation, lacerated basal plasma membranes, numerous lysosomes, and residual bodies) and showed significant degeneration of trypanosomes in these cells (51). The low incidence of flies with dual infections (0.02%) suggests that these flies suffer high mortality (51).

Co-infection of SGH-infected tsetse flies with rickettsia-like organisms (RLOs) was observed in two studies in the late 1980s (18, 50). Quantitative data are available from a small sample size ($n = 12$) and demonstrate a high incidence (83%) of RLO infection in *G. pallidipes* with SGH, suggesting that the presence of RLOs may increase susceptibility of salivary glands to viral infection or vice versa (18). It

is unknown if RLOs induce additional pathological effects in the enlarged, SGHV-infected salivary glands, nor is there any proven role of RLOs in predisposing tsetse flies to trypanosome infections. Based on current knowledge, these reported RLOs may represent bacterial symbionts associated with tsetse flies (7).

TRANSMISSION OF SGHV

Transmission of SGHVs is dictated by the biology of the host. Viral transmission in field and laboratory populations of the viviparous, hematophagous tsetse has been the subject of many studies (4, 34, 35, 39–41, 74–77), and two main routes have been suggested: (*a*) vertical transmission from mother to offspring and (*b*) horizontal transmission by oral infection through the gut. The presence of SGH in teneral (no blood meal yet taken) progeny of viremic females mated with normal males supports the hypothesis that the virus could be transmitted from mother to offspring (34), possibly by the transovarial/transovum route (62). The high SGHV genome copy numbers in pupae produced by females with SGH, compared to the low numbers in pupae produced by females with normal salivary glands, likewise support this hypothesis (4). The presence of virus particles within germarial cells, nurse cells, and oocytes of ovaries is an additional indicator of transovarial transmission (41). Virus particles present in milk glands suggest transmission from mother to offspring through oral ingestion of the milk by the larva developing in the uterus (74). Effective SGHV transmission from females with SGH to their progeny is hindered by their reduced fecundity (74), which is likely caused by malnutrition due to impaired feeding (77) and by necrotic lesions in ovaries (40, 41) and milk glands (76). In females that do produce offspring, vertical virus transmission to the F_1 is not an absolute outcome. For instance, only 21% and 48% of progeny produced by SGH-positive *G. m. centralis* and *G. m. morsitans*, respectively, developed SGH (74). A low transmission rate can explain the low prevalence of SGH in the field, but it is not clear which other mechanism exists that enables the virus to maintain itself in nature. Hypertrophied salivary glands of category 1 (0.1-mm diameter of distal part indicating an early stage of SGH) were found in very old flies, explained by latent infections originating from low doses of virus transmitted from mother to offspring or by adult tsetse flies acquiring the virus infection from the environment (62).

In a laboratory colony of *G. pallidipes* that was maintained using the membrane-feeding technique, a very high rate of SGH (85%) was due to horizontal transmission during membrane feeding (1). Jaenson (34) speculated on the possibility of horizontal transmission of SGHV, and Odindo et al. (65) demonstrated horizontal transmission by feeding a suspension of hypertrophied salivary glands to *G. pallidipes* teneral flies, of which 31% developed SGH. Moreover, one viremic fly can deposit 10^7 viral genome copies into the blood under the membrane during feeding, and the observation that these secreted viruses can initiate an infection in healthy tsetse flies confirms the horizontal transmission route (5).

Horizontal transmission is the major transmission route of MdSGHV in the gregarious house fly. During a few seconds of feeding, infected flies deposit an average 10^6 viral genome copies onto a solid food substrate. The virus released in the salivary secretions is highly infectious to newly emerged conspecifics, causing 66% infection rates in the challenged flies (55). There is evidence that the virus is acquired only during the adult stage; flies exposed as larvae to oral treatments of virus preparations do not develop SGH (24), whereas similar treatments of newly emerged adults result in average infection rates varying between 30% and 83% at 6–7 days posttreatment (55, 71). In addition, larvae collected from field sites with high incidence of SGH in adults (max. 34%) and reared to adulthood in the laboratory do not display SGH as seven-day-old adults (24). Mating experiments have demonstrated that MdSGHV is transmitted neither sexually between healthy and infected mating partners nor vertically to progeny (24, 54).

GEOGRAPHICAL DISTRIBUTION AND PREVALENCE OF SGHV

It is apparent that the narrow host range of SGHVs dictates their geographical distribution (**Table 1**). In the only sampled field populations of narcissus bulb flies in southern France, the prevalence of viral infection was high (>31%) (56). At present, no assumptions can be made about the geographical distribution of MeSGHV. Distribution of the tsetse SGHVs is restricted to the African continent and tied to the distribution patterns of the hosts. In *Glossina* spp., field incidence of SGH symptoms varies from as little as 0.08% (13) to as much as 15.6% (62). In contrast, the MdSGHV infecting house flies has a global distribution with average prevalence rates at individual sites ranging from 0.5% to 10% (**Table 1**).

Factors Influencing SGHV Dynamics in Natural Populations

Although flies with SGH can be found in most surveyed areas, the number of symptomatic flies varies by location and even by trap site (24, 62, 63, 67, 71). In addition, seasonal fluctuations in SGH prevalence were observed within trap sites (24, 62). There is evidence that both the ecosystem and fly density affect the prevalence of SGH. As expected with an orally transmitted disease, house fly density correlates positively with SGH incidence (24). On the other hand, prevalence of vertically transmitted tsetse SGHV is inversely correlated with host density (62). This inverse relationship suggests that the virus may be one of the population-regulating factors in the viremic populations. Only one study has attempted to identify climatic factors that impact field incidence of SGH (63). In a forest ecosystem in Kenya, prevalence of SGH was positively correlated with vegetation density and rainfall (humidity) and negatively correlated with temperature and age structure of the tsetse population (63). SGH is found in all age categories of the adult host (62). Although the diameter of hypertrophied salivary glands increases as flies age, glands with a high degree of hypertrophy (0.8-mm diameter) can be found in newly emerged flies and glands with a low degree of hypertrophy (0.1-mm diameter) can be found in aged flies (34, 62). These variations in disease expression could be due to different incubation periods of the vertically or horizontally transmitted virus, which may exist in an asymptomatic stage before unknown factors trigger expression of SGH. Alternatively, the host may have acquired the virus during an earlier life stage (likely to occur in tsetse flies but not in house flies) or as an older adult.

In several host populations, the incidence of SGH is higher in males than in females. In the narcissus bulb fly, for example, symptomatic SGH was recorded in 88% of males and 16% of females of *M. equestris* var. *nobilis* and in 93% of males and 30% of females of *M. equestris* var. *transversalis*, accounting for 5.5-fold- and 3.1-fold-higher incidence, respectively, in males than in females (56). In house fly and tsetse field populations, incidence of SGH may be up to 2-fold and 4.6-fold higher, respectively, in males than in females (24, 28). Similar observations were made in laboratory colonies of *G. pallidipes* (1).

Prevalence of SGH in Laboratory Tsetse Fly Colonies

Whereas the prevalence of SGH in wild tsetse flies is low (0.2–5.4%), with the exception of the high rate (15.6%) in Kenya as reported by Odindo (62), the prevalence of SGH in laboratory tsetse colonies varies greatly with colony and over time. The SGH prevalence in a *G. pallidipes* colony that originated from Tororo, Uganda, and was maintained at the Insect Pest Control Laboratory of the FAO/IAEA in Seibersdorf, Austria, varied between 3.8% in 2007 and 10% in 2008 and 2009, a prevalence low enough to enable maintenance of the colony. However, a *G. pallidipes* colony originating from the Southern Rift Valley in Ethiopia and established at IAEA, Austria, became extinct in 2002 as a result of high SGH prevalence (85%) (1). In a *G. pallidipes* colony of the same origin, maintained at Kaliti, Addis

IPM: integrated pest management

SIT: sterile insect technique

Ababa, Ethiopia, SGH rates up to 45% were observed in 2008 (5).

The majority of prevalence studies of SGH in field populations relied on visual detection of SGH in dissected flies. Hence, reports only reflect prevalence of the SGH symptom and not general SGHV infection. Recent findings have shown that the GpSGHV can exist in an asymptomatic stage. In laboratory colonies of *G. pallidipes*, symptomatic SGH can be as low as 3.8%, but PCR-based assays detected the virus in almost all tested flies (1). A search (TBLASTX) for putative GpSGHV mRNAs in the expressed sequence tag database of another tsetse fly species, *G. morsitans morsitans* (**http://old.genedb.org/genedb/glossina/blast.jsp**), revealed significant identities (64%–100%) to 12 GpSGHV ORFs, suggesting that these expressed sequence tags were derived from cDNA synthesized from mRNA from tsetse flies asymptomatically infected with GpSGHV. It can be anticipated that SGHV prevalence in field populations is significantly higher than the reported prevalence of SGH.

SIGNIFICANCE OF SGHV IN TSETSE AND HOUSE FLY HOSTS

Glossina spp.

The obligatory blood-feeding tsetse flies (Diptera: Glossinidae) are solely responsible for the cyclical transmission of *Trypanosoma* parasites, the causative agents of human African trypanosomosis (HAT) (or sleeping sickness) in humans and African animal trypanosomosis (AAT) (or nagana) in livestock (52). An estimated 60 million people and 45–50 million cattle live under the constant risk of contracting the disease (14, 79). AAT is considered the single greatest constraint to improved livestock production in sub-Saharan Africa, with estimated direct annual cattle production losses of USD $600 million to $1.2 billion (33) and an annual lost potential in livestock and crop production of USD $4.75 billion (12).

Although AAT and HAT are contained mostly through curative and prophylactic treatment with trypanocidal drugs, the sustainable removal of the vector theoretically remains the most desirable strategy to contain the two diseases (38, 52). A variety of efficient tsetse control tactics is available, which can be combined in an integrated pest management (IPM) approach: a strategy that is derived from the principle that favorable aspects of different control methods complement each other, making the limitations of each method less important (11, 19). Environmentally acceptable tsetse control tactics include stationary bait techniques (29), the live bait technique (10), the sequential aerosol technique (45), and the sterile insect technique (SIT) (66, 70, 87). Applying the control effort on an area-wide (AW) basis, i.e., against an entire tsetse population within a circumscribed area (46, 48, 85), has resulted in more sustainable control (17, 45, 80, 87) compared with localized IPM where the control effort was directed against only parts of the tsetse population (9).

In 1996, the government of Ethiopia embarked on such an AW-IPM program to remove *G. pallidipes* Austen from 25,000 km^2 in the Southern Rift Valley. After the collection of the entomological baseline data (86), it was decided that eradication should be the strategy, with SIT as the final eradication component. Colonies of the local *G. pallidipes* strain were established in Addis Ababa and in Seibersdorf, but serious difficulties were experienced during the adaptation process, with up to 85% of the colonized flies showing SGH (5). Such high prevalence rates have drastic consequences for the mass rearing and the efficiency of SIT.

SIT requires rearing large numbers of target species individuals, which can then be sterilized using ionizing radiation and released sequentially over the target area (47). Rearing tsetse flies is challenging in view of their low reproductive capacity and the lack of an artificial diet. A high prevalence of SGH in a fly colony entails a high proportion of sterile males and of females with reduced fecundity. Above a certain threshold, such a colony cannot sustain itself and will decline and eventually collapse.

For SIT to be successful, the released sterile male flies should be able to compete with the wild target population (68, 84). Male *G. pallidipes* with SGH symptoms show testicular degeneration and have lost most of their potential to transfer viable sperm. Even if these male flies could track and mate with virgin female flies in nature, the absence of sperm transfer would not contribute to the induction of sterility in the native population. From laboratory studies in small cages, it is known that a proportion of female *G. austeni* and *G. tachinoides* Westwood will accept multiple matings (83), and assuming the same is true for *G. pallidipes*, any subsequent mating of such a female with a normal, wild male will result in a fully fertile female fly.

Strategies to manage the virus in these *G. pallidipes* colonies will therefore be required to produce high-quality, sterile male flies. Promising results have been obtained to reduce the virus load by using new, clean blood for each cage of flies for each feeding opportunity, rather than using the same blood for several successive feeds as is normally practiced for tsetse colonies (5). The high cost of the clean feeding method probably prohibits its use for large-scale rearing, but the clean feeding system could be used to establish a seed colony with low virus load. This colony could then be transferred to a normal feeding system combined with various virus management strategies that reduce virus replication [i.e., antiviral drugs and/or RNA interference (RNAi) for virus-specific genes] or that block horizontal transmission by neutralizing the virus infection in the blood using specific virus antibodies.

Musca domestica

The house fly, a global pest of agricultural and public health importance, has been known since antiquity (89). The ability of the fly to exploit a vast range of patchily distributed and ephemeral organic larval substrates has enabled it to plague virtually any area where humans and their animals congregate. Adult flies pose nuisance problems to farmworkers and to neighboring residents, but the habit of adult flies to defecate and regurgitate on animal and human food led to the early recognition of their role as vectors of human and animal pathogens, especially those responsible for enteric diseases (32).

RNAi: RNA interference

Because adult house flies can consume only liquids, they must regurgitate fluids from the alimentary system onto solid food in order to consume it in liquid form. This behavior is an important element in the movement and transmission of SGHV as well as human pathogens. Indeed, recent concerns about foodborne human illnesses have led to renewed documentation of the role of house flies in spreading disease-causing organisms, especially *Escherichia coli*, *Shigella* spp., and *Salmonella* spp. (6, 31, 57, 61). Pathogen-carrying flies are commonly found around human and animal waste and landfills, from which they disperse to areas of human habitation and activity (58, 81).

Conventional management of house flies has relied on the use of residual insecticides applied to fly resting sites, pyrethrin space sprays, and sugar baits containing toxicants. The rapidity with which house flies develop high levels of resistance to residual insecticides is legendary and has made it exceedingly difficult to control flies in areas with long histories of the use of common toxicants such as permethrin and cyfluthrin (27, 78). Cross-resistance and the high innate tolerance of flies have led to surprisingly high levels of resistance to novel insecticides such as imidacloprid within a few years of their introduction, even when these toxicants are deployed as baits (44).

Other methods of fly management include cultural control, especially removal of manure and other breeding habitats, the use of various types of traps, and biological control. Most of the biological control efforts have targeted the immature stages of the fly, with main emphases on egg predators and pupal parasitoids (23, 72). In contrast, biological control options for adult house flies have received relatively little attention. The entomopathogenic fungus *Entomophthora muscae* often produces spectacular epizootics in house fly populations (60, 88), but attempts to manipulate this pathogen have

been limited by the need for high fly populations to sustain epizootics (25) and the ability of the flies to mitigate the effects of infection by resting in warm areas to raise their body temperature (43). Similarly, adult house flies are susceptible to *Beauveria bassiana* and *Metarhizium anisopliae* (16, 26, 53), but attempts to use these pathogens in the field have met with mixed results. MdSGHV is a particularly attractive candidate for fly biocontrol because it is already compatible with the ecology and behavior of the fly, and its ovary-suppressing effect is unique among natural enemies of the fly.

SUMMARY POINTS

1. SGHVs, unlike many other insect viruses, have unique pathological properties: They infect the adult stage, cause a chronic infection that produces little if any external symptoms, and at the cellular level cause a unique pathology in the salivary gland.
2. This virus group is novel and has been proposed to constitute a new virus family, the *Hytrosaviridae*.
3. The discovery of *Hytrosaviridae* is hindered due to the absence of obvious external symptoms and/or acute mortality associated with infection.
4. The ability of MdSGHV to downregulate vitellogenesis and disrupt mating behavior in the infected host provides for potential manipulation as a natural sterilizing control agent.

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